Journal of Surgical Oncology 1998;67:85–89

Morphologic and Mucin Histochemical Analysis of Transitional Zones in Advanced Ulcerated Colorectal Carcinomas: Potential Prognostic Indicators

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Background and Objectives: The transitional zone, which is normal-appearing mucosa that surrounds a primary colorectal carcinoma, has characteristic histologic features, and an increased amount of sialomucin in the transitional zone have been associated with a poorer prognosis. To clarify the prognostic effects of changes in the transitional zone we studied the transitional zone in cancers of the colon and rectum.
Methods: A total of 105 specimens resected for advanced colorectal carcinoma were studied to identify the effectiveness of evaluating morphologic types (polypoid or nonpolypoid growth type) and mucin expression (sulfomucin or sialomucin type) of the transitional zone as a prognostic indicator.
Results and Conclusions: Nonpolypoid carcinomas were likely to have invaded the deeper layers and lymphatic vessels and go on to develop advanced disease. Sulfomucin-type tumors were predominantly found in the right side colon and followed a relatively favorable course. Our results indicate that the morphologic and mucin components of the transitional zone may be prognostic indicators for advanced colorectal carcinoma.
J. Surg. Oncol. 1998;67:85–89.

KEY WORDS: morphology; mucin staining; transitional zone; advanced ulcerative colorectal carcinoma

INTRODUCTION

The transitional zone (TZ) is the otherwise normal-appearing mucosa that surrounds a primary colorectal carcinoma and exhibits both characteristic changes on morphologic examination and abnormal mucin production (a shift from sulfomucin to sialomucin) [1–4]. Although there is controversy concerning the cause of this change [5], many investigators have suggested that alterations in mucin production are specific indicators of a neoplastic or preneoplastic condition and that these changes positively relate to recurrence or prognosis [6–10].

The purpose of this study was to characterize morphologic features and mucin production at the TZ and to determine if these characteristics are prognostic indicators in patients with advanced, ulcerating colorectal carcinoma.

MATERIALS AND METHODS

A total of 105 patients with advanced ulcerating carcinomas of the colon and rectum who underwent resection in our department between 1980 and 1996 were studied. Advanced ulcerating carcinomas were defined as those grossly characterized by a large central and deep ulceration with a raised edge as well as histologic evidence of invasion beyond the submucosal layer. The

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Accepted 27 October 1997

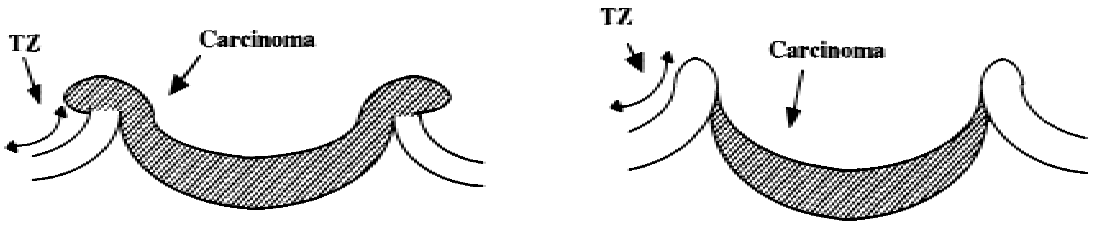

Fig. 1. The morphologic patterns of tumor growth. **Left:** polypoid growth type (PG-type cancer) characterized by an exophytic growth pattern with central ulceration. **Right:** nonpolypoid growth type (NPG-type cancer) primarily characterized by massive invasion into the deeper submucosal layers.

specimens were longitudinally sectioned from the central portion of the lesion to include the surrounding mucosa. The specimens were fixed in 10% neutral formalin, embedded in paraffin, sectioned 4 μm thick, and stained with hematoxylin and eosin (HE) and high iron diamine-alcian blue (HID-AB).

Transitional Zone

The TZ was defined as the mucosa surrounding the primary tumor within 1 cm of the ulcerated tumor margin.

Tumor Growth Pattern

According to Shimoda's classification [11], our tumors were classified into two groups based on the morphologic pattern of tumor growth as follows: (1) polypoid growth type (PG-type cancer) characterized by an exophytic growth pattern with central ulceration and a TZ covered by everted malignant mucosa at a central margin, and (2) nonpolypoid growth type (NPG-type cancer) primarily characterized by massive invasion into the deeper submucosal layers and central deep ulceration, and a TZ free of malignant mucosa (Fig. 1).

Mucin Histochemistry

All specimens were stained with HID-AB, which stains the cytoplasm of cells containing sulfomucin black and those with sialomucin blue. The number of mucosal cells present in individual crypts containing either the most sialomucin-staining cells or sulfomucin-containing cells were determined. Each histologic section was graded according to the largest percentage of sulfomucin- or sialomucin-stained cells. Sulfomucin-type cancers were those containing >50% of sulfomucin cells in the TZ, whereas the sialomucin-type cancers had >50% of sialomucin cells (Fig. 2).

TNM classification of malignant tumors according to the International Union Against Cancer (UICC) is used for staging system for colorectal carcinomas [12]. Comparison of different parameters was performed between these groups by the Chi square and Mann-Whitney U tests for nonparametric data, and the survival duration was calculated by the Kaplan-Meier method and statistical significance was assessed by the Mantel-Cox test.

RESULTS

There were 70 men and 35 women with an average age of 63.4 ± 12.7 years (range 32–92 yr). Overall, 72/105 (69%) carcinomas occurred in the right colon and 33/105 (31%) in the left colon. There were 26/105 (25%) stage I, 24/105 (23%) stage II, 35/105 (33%) stage III, and 20/105 (19%) stage IV tumors. Of the 105 carcinomas, 40 (38%) were of the PG-type cancer and 65 (62%) of the NPG-type cancer. Concerning mucin histochemical examination of the TZ, sulfomucin-type cancer was present in 21 (20%) cases and sialomucin-type cancer in 84 (80%) tumors (Table I). The relationships between the tumor growth type and clinicopathologic features are summarized in Table II. The location and frequency of PG-type cancer were similar to that of NPG-type cancer. Invasion to the serosa was evident in 22/40 (55%) cases of PG-type cancer and 51/65 (78%) of NPG-type cancer. There was a statistically significant difference in the incidence of invasion into the serosa based on tumor growth patterns ($P = 0.014$). Invasion to the lymphatic vessels was more likely to be found in NPG-type cancer (59/65; 91%) than in PG-type cancer (30/40; 75%) ($P = 0.029$). Of the PG-type cancer, 17/40 (43%) were stage I, 7/40 (18%) stage II, 10/40 (25%) stage III, and 6/40 (15%) stage IV. In the NPG-type cancer group, 9/65 (14%) were stage I, 17/65 (26%) stage II, 24/65 (37%) stage III, and 15/65 (23%) stage IV. The NPG-type cancer were of significantly advanced stage ($P = 0.039$). There were no differences in mucin composition or 5-year survival between PG-type cancer and NPG-type cancer.

The correlations between mucin staining and clinicopathologic features are shown in Table III. Of the sulfomucin-type cancer, 12/21 (57%) were located in the right side of the colon and 9/21 (43%) in the left side. Of the sialomucin-type cancer, 23/84 (27%) were found in the right colon and 61/84 (73%) in the left colon. Sialomucin-type cancer were more likely found in the left side of the colon than sulfomucin-type cancer ($P = 0.05$). There was no difference in the frequency of invasion to the

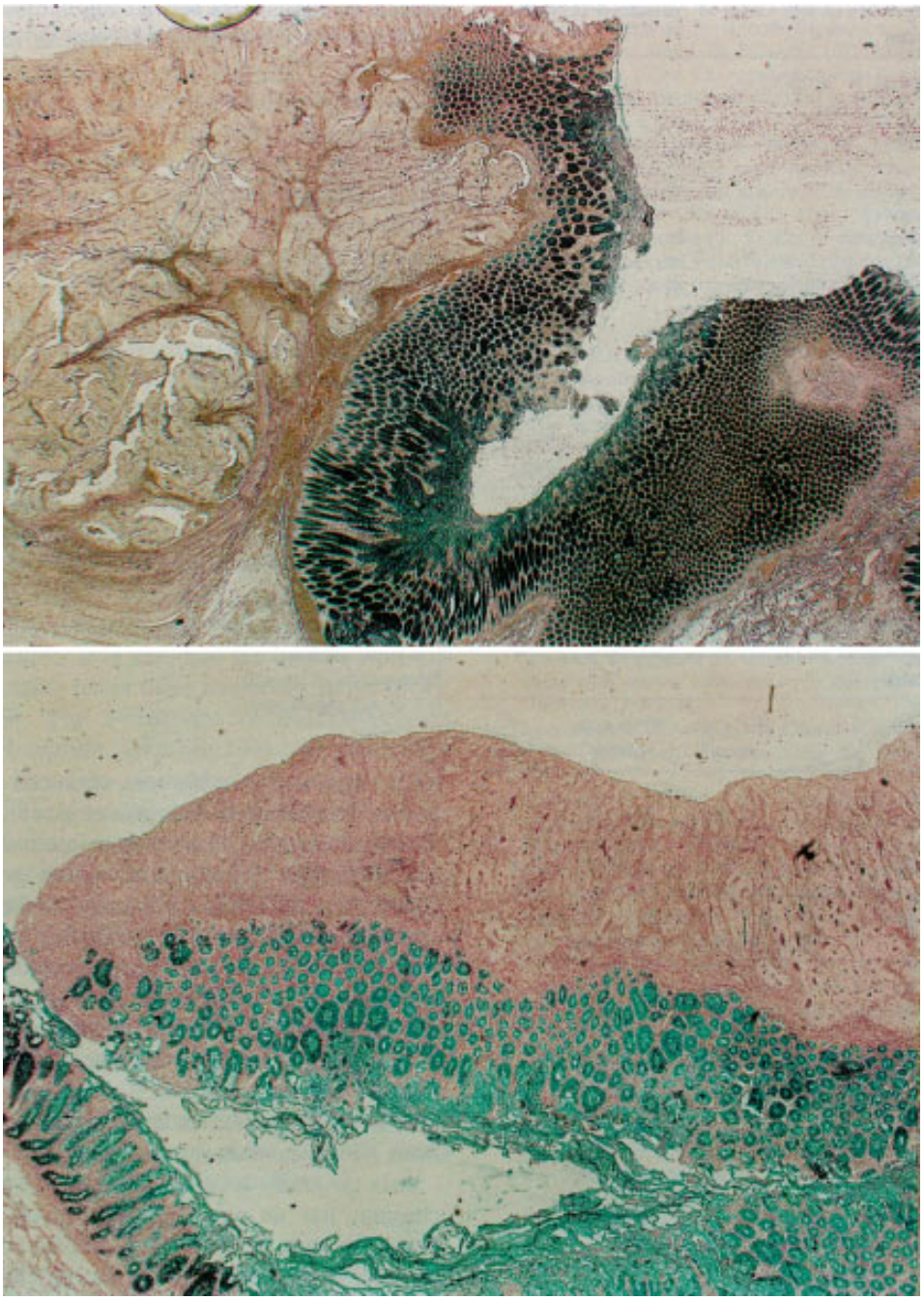

Fig. 2. Microscopic mucin profiles using high iron diamine-alcian blue (HID-AB) staining: sulfomucin-type cancer **(top)**, and sialomucin-type cancer **(bottom)**. (×25)

serosa or the lymphatic vessels based on mucin type. The tumor stage was similar in all groups. Sulfomucin was present in 11/21 (52%) cases of the PG-type cancer and in 10/21 (48%) of the NPG-type cancer. Similarly, sialomucin-type cancer was found in 54/84 (64%) of the PG-type cancer and in 30/84 (36%) of the NPG-type cancer. Five-year survival was significantly higher (91.7%) in the sulfomucin-type cancer group than in the sialomucin-type cancer group (62.4%) ($P = 0.05$). There was no correlation between the tumor growth type and the TZ mucin profile.

DISCUSSION

It is generally accepted that there are two pathways leading to the development of carcinomas of the human colon and rectum, specifically, the adenoma-carcinoma sequence and the ''de novo'' pathway. In clinical practice, most advanced colorectal carcinomas are ulcerated

TABLE I. Characteristics of 105 Cases of Advanced (TNM) Colorectal Carcinoma

Gender male:female = 70:35		
Age 63.4 ± 12.7 years		
Location in colon		
Right	72	(69%)
Left	33	(31%)
Stage (TNM staging)		
I	26	(25%)
II	24	(23%)
III	35	(33%)
IV	20	(19%)
Tumor growth patterns		
PG[a]-type cancer	40	(38%)
NPG[b]-type cancer	65	(62%)
Mucin type in TZ[c]		
Sulfomucin-type cancer	21	(20%)
Sialomucin-type cancer	84	(80%)

[a]PG, Polypoid growth.
[b]NPG, Nonpolypoid growth.
[c]TZ, Transitional zone.

TABLE II. Relationship Between Tumor Growth Pattern and Clinicopathologic Features in 105 Cases of Advanced (TNM) Colorectal Carcinoma

	PG[a]-type cancer	NPG[b]-type cancer	*P* value
Location in colon			
Right	15 (38%)	20 (31%)	
Left	25 (62%)	45 (69%)	.59 (NS)
Invasion to the serosa	22 (55%)	51 (78%)	.014
Invasion to the lymphatic vessels	30 (75%)	59 (91%)	.029
Stage (TNM staging)			
I	17 (43%)	9 (14%)	
II	7 (18%)	17 (26%)	
III	10 (25%)	24 (37%)	
IV	6 (15%)	15 (23%)	.039
Mucin type in TZ[c]			
Sulfomucin-type cancer	11 (28%)	10 (15%)	
Sialomucin-type cancer	29 (72%)	55 (85%)	.14 (NS)
5-year survival	71.3%	61.2%	.34 (NS)

[a]Polypoid growth.
[b]Nonpolypoid growth.
[c]Transitional zone.
NS = not significant.

TABLE III. Relationship Between Mucin Expression in TZ* and Clinicopathologic Features in Advanced Colorectal Carcinoma (TNM) (N = 105)

	Sulfomucin-type cancer	Sialomucin-type cancer	*P* value
Location in colon			
Right	12 (57%)	23 (27%)	
Left	9 (43%)	61 (73%)	.05
Invasion to the serosa	15 (71%)	58 (69%)	.82 (NS)
Invasion to the lymphatic vessels	18 (86%)	73 (87%)	.25 (NS)
Incidence of liver metastasis	2 (10%)	14 (17%)	.22 (NS)
Stage (TNM staging)			
I	4 (19%)	22 (26%)	
II	6 (29%)	18 (22%)	
III	6 (29%)	27 (32%)	
IV	5 (24%)	17 (20%)	.64 (NS)
Tumor growth type			
PG[a]-type cancer	11 (52%)	54 (64%)	
NPG[b]-type cancer	10 (48%)	30 (36%)	.15 (NS)
5-year survival	91.7%	62.4%	.05

*Transitional zone.
[a]Polypoid growth.
[b]Non-polypoid growth.
NS = not significant.

and contain a raised, everted edge. Recent advances in radiology and endoscopy have made it possible to detect early small, nonpolypoid colorectal carcinomas that are not associated with adenomas and massively infiltrate submucosal tissue. In these apparent de novo carcinomas, both the gross and histologic tumor growth patterns are identical to those of advanced nonpolypoid carcinomas (NPG-type cancer). Specifically, both are characterized by large and deep ulcers that form a surrounding protrusion as a result of proliferation of tumor cells in the deeper layers. Additionally, Nakamura et al. [13] have suggested that 70% of colorectal carcinomas develop from normal, flat mucosa, invade deeper tissue at an early stage, and then become advanced ulcerating carcinomas. Furthermore, Shimoda et al. [11] have classified advanced ulcerating carcinomas into two types, PG-type and NPG-type cancers. Even when grossly similar in appearance, advanced carcinomas of the NPG-type cancer had a poorer outcome, reflecting a more aggressive biologic behavior of these nonpolypoid de novo carcinomas occuring at an earlier stage than PG-type cancer.

In addition, the TZ of PG-type cancer is covered by malignant mucosa, whereas that of NPG-type cancer is free of malignant mucosa. Careful histologic evaluation of the TZ is helpful in differentiating PG-type cancer from NPG-type cancer.

It is generally accepted that the TZ of colorectal carcinomas has an abnormal pattern of mucin staining. These findings were shown in human colonic mucosa in carcinoma as well as in rats during experimental carcinogenesis [1–4]. However, there is controversy concerning the implications of increased sialomucin staining. Some investigators have claimed that this change represents a preneoplastic condition, whereas others have noted sialomucin staining in the mucosa adjacent to obviously nonneoplastic lesions, suggesting that the mucin may arise as a secondary phenomenon rather than as a specific change related to formation of colorectal carcinomas [5,9,10]. Experimental studies have shown that sialic acid residues present on mucin and other glycoproteins may play a role in the metastatic process [14,15]. The present study attempts to clarify the clinical significance of abnormal sialomucin production in the TZ sur-

rounding advanced ulcerating colorectal carcinoma. The main objective of this series is to determine if these mucin abnormalities can be used as a predictor of recurrence or survival in patients with advanced colorectal malignancies. Our results sugest that the sialomucin-type cancer was relatively more often in advanced ulcerating colorectal carcinomas than in polypoid carcinomas (not statistically significant), tended to occur in the left side of the colon, and was likely to develop synchronous or metachronous liver metastasis with a subsequent unfavorable prognosis.

It is unclear why the localization and biologic behavior of sialomucin-type cancer cells are different from those of sulfomucin-type cancer. Data in the literature suggest that sulfomucin reactive cells are inversely related to the severity of dysplasia [9,10]. Thus abnormal increased sialomucin production may strongly correlate with the degree of aggressive biologic behavior. Furthermore, the increased frequency of sialomucin-type cancer in the left side of the colon may be related to an increase in background susceptibility, or to longer exposure times to carcinogenic substances. It is said that the tumors with microsatellite instability occur more frequently in the proximal colon [16]. The difference of carcinogenesis containing microsatellite instability may bear some relationship to the alteration of mucin components in TZ. This issue should be resolved with further investigations.

Sialomucin is more prominent in metastases, and the mucin appears to increase the ability of malignant cells to adhere to endotherial cells [14]. In cell-line experiments, mucin production by human colon cancer cells correlates with their metastatic potential and affect their ability to colonize the liver [14,15]. Consequently, cells of sialomucin-type cancer may more easily disassociate from primary lesions and metastasize to target organs [15,17–19].

In conclusion, our results indicate that the histologic and mucin histochemical features of the TZ may play a role in predicting metastasis and prognosis.

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